A CORRELATIVE STUDY OF THE EXPERIMENTAL CARDIAC DYNAMICS AND MYOCARDIAL ENERGY METABOLISM

Taro Ishiyama, M.D., Yoshiharu Morita, M.D., Nozomu Tsukamoto, M.D., and Yuichi Yamamura, M.D.

Relationship between cardiac dynamics and myocardial energy metabolism was studied using dogs treated by isoproterenol, dinitrophenol, propranolol or amobarbital. Isoproterenol changed cardiac dynamic state to positive chronotropism with positive inotropism and myocardial energy liberation to uncoupling of oxidative phosphorylation. Dinitrophenol inducing uncoupling of oxidative phosphorylation, revealed also positive chronotropic and positive inotropic state. Propranolol changed cardiac dynamic state to negative chronotropism with negative inotropism, and myocardial energy liberation to suppression of oxidation. Amobarbital inducing inhibition of intracellular terminal oxidation, revealed also negative chronotropic and negative inotropic state. From the above-mentioned results, it might be clear that the positive chronotropism with positive inotropism appears to be relating to the uncoupling of oxidative phosphorylation, as well as the negative chronotropism with negative inotropism to the suppression of mitochondrial respiration.

Heart is beating with performing a tremendous work. Thus the energy metabolism is essential in myocardial metabolism. Many reports have been published on studies of oxygen and substrate supply and efflux through myocardial cell membrane. Subsequently, subcellular oxidation coupled with phosphorylation is also the essential process of energy liberation, which is performed in mitochondria. Up to date, the authors have studied on mitochondrial respiration of canine heart muscle using a polarographic method developed by Higihara. Among several experimental procedures, acute coronary occlusion revealed uncoupling of oxidative phosphorylation within one hour in dogs. Moreover, it was clarified that the uncoupling of oxidative phosphorylation was also revealed by ischemic hearts produced by other methods when the hearts were forced to continue beating, although such a phenomenon was not observed in ischemic hearts remained without forced beating. Therefore, it is obvious that the deteriorating factors of coupling of oxidative phosphorylation in mitochondria are ischemia with beating. In this study it was intended to investigate whether or not the stimulation or suppression of beating, that is, the increased or decreased external cardiac work, affects to myocardial intracellular energy liberation. Reversely another aim of this study is to confirm

Key Words:
- Mongrel dogs
- Isoproterenol-dinitrophenol
- Propranolol-amobarbital
- Chronotropic-inotropic diagram
- Chronotropic-inotropic vector
- Mitochondrial respiration-polarographic method
- Uncoupling of oxidative phosphorylation
- Inhibition of terminal oxidation

(Received on April 15, 1975; Accepted for publication on October 2, 1975)
The 3rd Department of Internal Medicine, Osaka University Medical School, Osaka
A part of this paper was read at the IX Interamerican Congress of Cardiology held in San Francisco in April, 1972)


1313
the effects of primary metabolic disorders to cardiac dynamics. Consequently, in this study it was intended to detect energy liberation in various cardiac dysfunctions produced by drugs. It is a purpose of this study to detect any relationship between cardiac dynamics and myocardial energy metabolism.

MATERIAL AND METHODS

Healthy adult mongrel dogs were used for this study, anesthetized by 20-35 mg/kg of pentobarbital soda. Dogs were divided into the following four groups, that is, isoproterenol group, dinitrophenol group, propranolol group and amobarbital group.

In each experimental group, the individual drug was injected intravenously. Isoproterenol was infused 0.2 µg/kg/min for about 10 minutes. Dinitrophenol was infused 0.3 mg/kg/min for 20 minutes. Propranolol was infused 0.1 mg/kg. Amobarbital was infused 3.5 mg/kg/min for 15 minutes. Before, during and after the drug administration, electrocardiogram, left intraventricular pressure and the first differential of left ventricular pressure (dp/dt) were monitored simultaneously and recorded sequentially by the polygraph EMR-100R of the Fukuda Electronics Co. ECG was principally observed by the II lead. Left intraventricular pressure was obtained by Hamilton No.8F catheter via the right carotid artery, transduced by the Statham P23Db strain gauge, and amplified by a carrier amplifier. dp/dt was electrically obtained by the time constant of 0.03 second. Paper speed was set to 10 cm/sec. Heart rate was calculated by the R-R interval of ECG. The isometric time tension index (ITTI, dp/dt/IT) by Siegel and Sonnenblick3) was calculated from ECG, left ventricular pressure and dp/dt.

In order to elucidate the cardiac dynamic change, a vectordiagram called “chronotropic-inotropic diagram” was constructed using the two parameters, heart rate (as an indicator of chronotropic) and ITTI (as an indicator of inotropic). O-point represented the initial state of the experiment. The X-axis represented percent changes of heart rates and the Y-axis represented percent changes of ITTI.

Time course of chronotropic and inotropic was plotted on this diagram and these points were called “chronotropic-inotropic points”. The straight lines connecting from the O-point to the chronotropic-inotropic points were called “chronotropic-inotropic vectors”.

In each experimental group, dogs were sacrificed after 60 minutes and the hearts were extirpated. Mitochondria were isolated with cell fractionation method from the minced left ventricular muscles. Details of measurement of the mitochondrial respiration have been described previously. Oxygen consumption rates were measured polarographically by Hagihara’s method. Glutamate was used as a substrate. The oxygen consumption rate in the state 3 (QO2 state 3, where the added phosphate acceptor, adenosine diphosphate (ADP), was converting to adenosine triphosphate (ATP) through oxidative phosphorylation in mitochondria), and the oxygen consumption rate in the state 4 (QO2 state 4, where the added ADP had been converted to ATP) were measured and calculated in nanoatoms of oxygen per milligram of mitochondrial protein per minute. The respiratory control index which was expressed by the division of QO2 state 3 by QO2 state 4 (indicating the tightness of coupling of oxidative phosphorylation) and the ADP/O ratio which was expressed by the quotient of moles of added ADP to atoms of consumed oxygen in the state 3 were calculated.

RESULTS

I. Cardiac dynamic studies

Figure 1 shows an example of cardiac dynamic changes before, during and after the intravenous infusion of isoproterenol. Heart rate


- Fig.1. An example of cardiac dynamic changes during and after infusion of isoproterenol.
  HR: heart rate,
  LVSP: left ventricular systolic pressure,
  dp/dt max: maximum value of the first differential of left ventricular pressure,
  ITTI: isometric time tension index.
increased immediately. Left ventricular systolic pressure (LVSP) and maximum dp/dt (dp/dt max) decreased tentatively just after the start of infusion. Meanwhile, ITTI did not show any dip but elevated gradually.

Figure 2 shows an example of cardiac dynamic changes before and during intravenous infusion of dinitrophenol. Heart rate increased gradually. LVSP showed no remarkable change but dp/dt max increased gradually. Thus ITTI increased gradually.

Figure 3 shows an example of cardiac dynamic changes before and after intravenous injection of propranolol. Heart rate decreased moderately and LVSP, dp/dt max and ITTI also decreased remarkably.

Figure 4 shows an example of cardiac dynamic changes before and after intravenous infusion of amobarbital. These changes were very similar to those of propranolol as shown in Figure 3.

Figure 5 shows vectordiagramic represen-
Fig. 5. Vector-diagnostic presentations of the cardiac dynamic changes of the examples shown in Fig. 1, Fig. 2, Fig. 3, and Fig. 4. The central unit figure represents the legend. The X-axis represents chronotropic effect and the Y-axis represents inotropic effect, expressed by the percent changes of the initial values of heart rate and ITTI.

Fig. 6. The maximum chronotropic-inotropic vectors of individual experimental cases. These vectors were derived from the individual chronotropic-inotropic diagrams. Each vector represents one experimental case.

Fig. 7. Changes of mitochondrial respiration. The four experimental groups and a control group with intact left ventricles were illustrated (mean ± standard error). In each group, the first left black column represents the oxygen consumption rate in the state 3 (O₂ state 3), the next grey column represents the oxygen consumption rate in the state 4 (O₂ state 4), the third hatched column represents the respiratory control (RC) and the fourth open column represents shows the ADP/O ratio (P/O).

O₂ state 4 was significantly lower. Thus respiratory control index was around 5.5. In the isoproterenol group, O₂ state 3 decreased (p < 0.001) and O₂ state 4 increased (p < 0.001). Therefore the respiratory control index was markedly deteriorated (p < 0.001). ADP/O ratio was unchanged. These changes were similar to those of the dinitrophenol group. This pattern of changes in mitochondrial respiration suggested the uncoupling of oxidative phosphorylation.

In the propranolol group, both O₂ state 3 (p < 0.001) and state 4 (p < 0.05) decreased. Although the respiratory control index was somewhat deteriorated because the rate of decrease in O₂ state 3 was somewhat greater than in O₂ state 4, the level of the respiratory control index was substantially same with the normal control group. ADP/O ratio was unchanged. These changes were similar to those of the amobarbital group. This pattern of changes suggested the suppression of mitochondrial respiration.

III. Relationship between cardiac dynamic changes and disorders of mitochondrial respiration

According to the above-mentioned results, Figure 8 was summarized. Isoproterenol induced positive chronotropic and positive inotropic action primarily, resulted decrease in O₂ state 3 and increase in O₂ state 4, thus the respiratory control index was deteriorated. On the contrary, dinitrophenol primarily affected to the coupling of oxidative phosphorylation, resulting in a positive chronotropic and positive inotropic effect to the heart. Therefore as shown in Figure 9, it appears to be suggestive that the first quadrant of the chronotropic-inotropic diagram was the corresponding area of disturbance of energy liberation by the uncoupling of oxidative phosphorylation.

As shown in Figure 8, when negative chronotropism with negative inotropism was induced by propranolol, both O₂ state 3 and O₂ state 4 decreased, thus the respiratory control index was substantially remaining unchanged. And, when the suppression of mitochondrial oxidation was primarily induced in the respiratory chain by amobarbital, both chronotropism and inotropism tended to be negative. Therefore as shown in Figure 9, the third quadrant appears to be the corresponding area of disturbance of energy liberation by suppression of mitochondrial respiration.
lation of cardiac dynamics and myocardial energy metabolism. Sarnoff et al.\(^4\) advocated the concept of tension time index (TTI) and emphasized that the TTI was a sharp indicator of the amount of myocardial oxygen consumption. Katz\(^5\) described an index to estimate the amount of oxygen consumption in correlation with heart rate and blood pressure. It seemed to be, however, that recent advances in cardiology research have proposed several important re considerations in the above-mentioned concepts. This paper intended to find out a more detailed relationship between cardiac dynamics and myocardial energy metabolism.

The first controversy against the above-mentioned concepts is that mechanical energy required by beating is essentially utilized to develop isometric tension and it amounts for about seven to ten times of energy required for the external cardiac work.\(^6\) Then it seems to be meaningless to correlate TTI to myocardial oxygen consumption. Siegel and Sonnenblick proposed two indicators, that is, the isometric time tension index (ITTI, dp/dt/ITT)\(^6\) and the maximum fiber shortening velocity (Vmax)\(^7\) to evaluate developed tension in the isometric contraction phase of a ventricle. At present, in order to evaluate cardiac dynamics it is believed to be reasonable to prefer an index of myocardial contractility to external cardiac work. Another problem is based on the lack of knowledge concerning to the subcellular respiration in myocardial energy metabolism. However, the process of energy liberation is more important than oxygen supply. Even if oxygen supply is sufficient, oxygen may be wasted without coupling of phosphorylation to oxidation in some pathologic states of myocardium. Therefore it is necessary not only to detect oxygen supply but to observe subcellular respiration in order to disclose myocardial energy metabolism in various cardiac dysfunctions. According to the above-mentioned considerations, ITTI was measured as an index of inotropism in the isometric contraction phase, and the mitochondrial respiration was evaluated as an indicator of myocardial energy metabolism. It is unquestionable that an index of chronotropism is equally important to evaluate cardiac dynamic states. Therefore heart rate was carefully noticed in this study. In convenience to express changes of heart rate and contractility simultaneously, a vectordiagraphic method of presentation was devised.\(^8\) It was called

---

**Fig. 8.** Relationship between cardiac dynamic changes and disorders of mitochondrial respiration, illustrated on the chronotropic-inotropic diagram.

**Fig. 9.** Relationship between cardiac dynamics and myocardial energy metabolism.

---

**DISCUSSION**

Many investigations have been published concerning to the studies of cardiac dynamics as well as myocardial energy metabolism. However few studies were seen on the corre-
"chronotropic-inotropic diagram". As it is clear in the results, changes of dynamic states of the heart were visualized by this vectordiagraphic presentation.

In this study four drugs were used to induce cardiac dysfunctions in which any relationship between cardiac dynamics and myocardial energy metabolism was to be observed, since these drugs successfully induced variations of either cardiac dynamic state or energy metabolism.

In the isoproterenol group, a few cases showed transient fall in LVSP and dp/dt as shown in the figure. This might be due to the vasodilating action of isoproterenol. It dilates peripheral arteries and decreases afterload to left ventricular ejection, resulted fall in LVSP and dp/dt. However, at the same time ITTI did not fall down. This fact strongly suggests evidence that the ITTI is not influenced by afterload and is an excellent indicator of cardiac contractility as Siegel and Sonnenblick pointed out[5]. Isoproterenol induced positive chronotropic and positive inotropic effects to the hearts as expected. In the aspects of energy liberation, myocardial mitochondrial respiration revealed uncoupling of oxidative phosphorylation. Isoproterenol are known to injure myocardium in a large amount. But it is unlikely that such a small doses as has been used in this study could possibly injure myocardium directly. Therefore the observed uncoupling of oxidative phosphorylation appears to be attributed to the increase in heart rate and contractility. Dinitrophenol is known to be an uncoupler of oxidative phosphorylation in vitro, and affects the respiratory chain at the three sites of phosphorylation coupled with oxidation as shown in the figure 10. It appeared to be possible that the uncoupling of oxidative phosphorylation was induced in vivo when this drug was administered. In these occasions, a question should be arisen whether positive chronotropism with positive inotropism could be induced by dinitrophenol as same in cases of isoproterenol. Dinitrophenol presented the results as predicted. So the observed positive chronotropism with positive inotropism was corresponded to the uncoupling of oxidative phosphorylation.

In both the isoproterenol group and the dinitrophenol group, ADP/O ratios were unchanged, even though respiratory control index was deteriorated. Respiratory control index is believed to be a more sensitive indicator of coupling of oxidative phosphorylation than ADP/O ratio.

Amobarbital is a well known inhibitor of oxidation in the electron transfer system in vitro. The site of inhibition is shown in the figure 10. It was suspected that the suppression of oxidation might be induced with this drug was administered to dogs. In these occasions it is interesting whether or not negative chrono-
tropism with negative inotropism might be induced similarly as in cases of the propranolol group. Amobarbital revealed the predicted results.

Therefore as described in the results, a certain relationship was evident phenomenologically between cardiac dynamic changes and myocardial energy metabolism. At present this relationship would be admitted only when the above-mentioned drugs were used to change either cardiac dynamic state or energy metabolism. It is believed to be essential in the near future to clarify whether or not such a relationship between cardiac dynamics and myocardial energy metabolism is evident in other causes of cardiac dysfunctions.

REFERENCES